ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., 72 (3), September 2020: 439-454

Aquatic Ecology
Research Article

Published online 05 May 2020
http://www.acta-zoologica-bulgarica.eu/002370

The Balkan Macrophyte Index (BMI) for Assessment of Eutrophication in Lakes

Susanne C. Schneider[1*], Sonja Trajanovska[2], Vera Biberdžić[3], Aleksandra Marković[4], Marina Talevska[2], Alma Imeri[5], Elizabeta Veljanoska-Sarafiloska[2], Pavle Đurašković[6], Katarina Jovanović[7] & Magdalena Cara[5]

[1] Norwegian Institute for Water Research, Gaustadalleen 21, 0349 Oslo, Norway
[2] Hydrobiological Institute Ohrid, Naum Ohridski 50, MK-6000 Ohrid, North Macedonia
[3] Natural History Museum of Montenegro, Vojvode Bećir-bega Osmanagića 16, P.O.Box 374, Podgorica, Montenegro
[4] Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, Belgrade, Serbia
[5] Agricultural University of Tirana, Koder–Kamez, 1029 Tirane, Albania
[6] Institute of Hydrometeorology and Seismology, 4th Proleterske brigade 19, Podgorica, Montenegro
[7] Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", Dr Subotica 5, 11000 Belgrade, Serbia

Abstract: Aquatic plants have long been used as indicators for nutrient enrichment in lakes. In the Mediterranean, however, the process of developing and intercalibrating macrophyte assessment methods for lakes has lagged behind Northern and Central Europe, likely due to the relatively small number and high variability of natural lakes in the Mediterranean but also because of the different monitoring traditions in different parts of Europe. We here present a macrophyte index for assessment of lake eutrophication, tailored to Balkan lakes (Balkan Macrophyte Index, BMI). We analysed submerged aquatic vegetation, water chemistry and sediment total phosphorus content at several sites in lakes Prespa, Ohrid, Lura, Biogradsko, Crno and Sava, located in Albania, North Macedonia, Montenegro and Serbia. Despite the restricted number of lakes in our dataset, the BMI was loosely related to water phosphorus, rather than nitrogen, concentrations. Our results show that macrophyte indices may not be applicable in lakes experiencing annual water level fluctuations of several meters, because the macrophyte vegetation in such lakes may be absent, or alternatively dominated by "oligotrophic" or "eutrophic" species. Once a larger number of lakes has been analysed using the same methods, reference conditions and status class boundaries may be derived from the phosphorus – BMI regression.

Key words: Macrophytes, eutrophication, Water Framework Directive, phosphorus, indicator value

Introduction

Eutrophication still is considered to be the most widespread stressor in lakes in Europe (Noges et al. 2016). Clean lakes provide safe drinking water, protect human health, support economic and recreational activities, and provide healthy habitats for flora and fauna. Therefore, regular monitoring of lake water quality is important. Monitoring chemical compounds in lake water, such as total phosphorus concentration, provides snapshot information on specific parameters of concern. Ecological

* Corresponding author: susi.schneider@niva.no

assessment of lakes complements chemical monitoring because biota provide a longer-term insight into prevailing conditions than chemical measurements, and because living elements may respond to multiple stressors within an ecosystem (Karr 1999). Aquatic plants are considered useful as indicators for what has been termed "ecological status" in Europe (EC 2000) and "ecosystem health" or "biotic integrity" elsewhere (Karr 1999). Macrophytes and phytobenthos are mandatory elements in status assessment of lakes, as required by the Water Framework Directive (WFD; EC 2000), along with phytoplankton, benthic invertebrates, and fish. In the last years, hundreds of assessment systems were developed and tested in Europe (Birk et al. 2012), among them almost 20 focusing on the use of aquatic plants for the assessment of eutrophication in lakes (Poikane et al. 2015). The WFD also triggered an intercalibration process, i.e. the harmonization of monitoring and assessment methods across countries and ecoregions (Poikane et al. 2015). Therefore, ecological status classes are now comparable across Europe.

In the Mediterranean, however, the process of developing and intercalibrating macrophyte assessment methods for lakes has lagged behind Northern and Central Europe. This was due to the relatively small number and high variability of natural lakes in the Mediterranean compared to Northern and Central Europe but it also reflects different monitoring traditions in different parts of Europe (Birk et al. 2012, Poikane et al. 2015, Zervas et al. 2018). Kolada et al. (2014) developed a macrophyte metric, which was aimed at being applicable to assess eutrophication in lakes across Europe. However, the metric performed better in Nordic than in Central-Baltic lakes, and no lakes from the Mediterranean were included. Consequently, the applicability of this metric in the Mediterranean is uncertain. Recently, however, a macrophyte index for monitoring and assessment of Greek lakes was published (Zervas et al. 2018). Its indicator values are based on the values given in Kolada et al. (2014), and the index seemed to perform well in Greece. However, no methods for assessment of macrophytes in lakes have been adopted by Western Balkan countries yet.

First attempts of applying a macrophyte index for eutrophication in the Western Balkans were made in Lake Ohrid (Trajanovska et al. 2014) and Lake Prespa (Trajanovska et al. 2019). Here, the "macrophyte index" (MI; Melzer 1999, Melzer & Schneider 2001), developed in South Germany, was successfully applied, and produced meaningful results. However, the application of assessment methods across countries or ecoregions is not always straightforward. It has been shown that indicator values of macrophyte species for assessment of eutrophication are broadly similar across Central Europe (Schneider 2007). However, the species inventory may differ between different geographic regions, and consequently, if an index developed in one region is applied to another, potentially informative species may not be used (Schneider 2007). E. g., endemic species occur in Balkan lakes (Blaženčić et al. 2006, Albrecht & Wilke 2008) and their potential bioindicator value should not be neglected *a priori*.

The aim of our project was, therefore, to develop a macrophyte index for assessment of lake eutrophication, tailored to Balkan lakes. We aimed at broad applicability in the Western Balkans and, therefore, performed a collaborative effort among four countries: Albania, North Macedonia, Montenegro and Serbia.

Materials and Methods

Sampling sites

We sampled six lakes in the Western Balkans (Fig. 1): a) Lake Prespa – a transboundary lake situated between Albania, North Macedonia and Greece; surface area: 254 km², max depth 58 m, average depth 14 m (Matzinger et al. 2006); b) Lake Ohrid – a transboundary lake situated between Albania and North Macedonia; surface area 358 km²; max depth 289 m, average depth 155 m (Matzinger et al. 2006); c) Lake Lura – Albania; surface area 0.12 km², max depth 20 m, no information exists

Fig. 1. Map of sampled lakes.

Table 1. Location of sampling sites and summary statistics of sediment total phosphorus content, water total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD). All values are averages from two measurements performed in spring and autumn, except numbers marked in italics, where only autumn samples were taken.

Lake	Site	Name	Latitude	Longitude	Sediment TP (mg/kg)	Water TN (µg/l)	Water TP (µg/l)	Water BOD (mg/l)
Prespa	LP1	Stenje	40° 56’ 32.4636”	20° 54’ 41.5656”	266	512	29	1.4
	LP2	Konjsko	40° 54’ 39.1176”	20° 59’ 24.5364”	532	546	25	2.2
	LP3	Golomboh	40° 51’ 31.1832”	20° 56’ 48.624”	490	588	25	1.5
	LP4	Pustec	40° 47’ 29.5512”	20° 54’ 47.1852”	549	589	22	4.6
	LP5	Krani	40° 57’ 13.7088”	21° 4’ 14.3436”	211	492	23	1.8
	LP6	Sirhan	40° 59’ 7.746”	20° 55’ 12.3996”	391	526	26	2.7
Ohrid	LO1	Kalishta	41° 9’ 1.5516”	20° 39’ 4.482”	310	306	8	1.2
	LO2	Ohrid bay	41° 6’ 42.3864”	20° 47’ 53.8908”	1280	470	7	1.3
	LO3	Velidab	40° 59’ 12.0984”	20° 47’ 53.6136”	244	270	5	1.2
	LO4	Trpejca	40° 57’ 45.0576”	20° 47’ 0.7908”	333	376	6	1.6
	LO5	Tushemisht	40° 54’ 23.058”	20° 43’ 29.964”	176	796	9	1.5
	LO6	Lini	41° 4’ 3.1368”	20° 38’ 35.8332”	471	377	9	1.5
Lura	LL1	Big Lake 1	41° 47’ 26.53”	20° 11’ 46.25”	230	405	8	0.9
	LL2	Big Lake 2	41° 47’ 23.16”	20° 11’ 46.25”	191	232	9	0.9
	LL3	Big Lake 3	41° 47’ 15.3”	20° 11’ 34.4”	470	403	10	0.8
	LL4	Big Lake 4	41° 47’ 20.0”	20° 11’ 29.9”	*218*	*231*	*11*	*2.2*
	LL5	Big Lake 5	41° 47’ 28.9”	20° 11’ 31.4”	*205*	*228*	*20*	*0.8*
	LL6	Big Lake 6	41° 47’ 27.5”	20° 11’ 39.4”	*134*	*236*	*12*	*1.2*
Biogradsko	LB1	Camp	42° 54’ 1.62”	19° 35’ 45.28”	*2340*	652	20	2.8
	LB2	NW bay	42° 54’ 2.26”	19° 35’ 49.77”	*390*	539	16	1.5
	LB3	N bay	42° 54’ 0.23”	19° 35’ 56.36”	*1930*	677	16	0.9
	LB4	NE bay	42° 53’ 55.28”	19° 36’ 1.37”	*1270*	519	23	2.7
	LB5	SE bay	42° 53’ 52.08”	19° 35’ 58.95”	*1760*	456	17	2.1
	LB6	SW bay	42° 53’ 55.77”	19° 35’ 51.14”	*1230*	580	18	2.3
Crno	LC1	Mlinski potok	43° 8’ 51.43”	19° 5’ 16.50”	*450*	678	20	1.5
	LC2	NE coast	43° 8’ 55.85”	19° 5’ 35.01”	*600*	593	22	1.7
	LC3	Restaurant	43° 8’ 49.47”	19° 5’ 45.52”	*400*	682	14	2.3
	LC4	Splav	43° 8’ 39.54”	19° 5’ 37.49”	*960*	503	16	2.1
	LC5	NW bay - Malo j.	43° 8’ 40.9”	19° 5’ 6.77”	*1060*	605	13	1.4
	LC6	E coast - Malo j.	43° 8’ 34.15”	19° 5’ 27.82”	*3210*	466	13	1.4
Sava	LS1	Markoni	44° 47’ 13.247”	20° 24’ 39.888”	248	430	9	1.7
	LS2	Zip line	44° 47’ 0.384”	20° 23’ 37.615”	189	395	6	1.7
	LS3	Plaža	44° 46’ 49.973”	20° 23’ 4.110”	231	425	7	1.7
	LS4	nudist beach	44° 46’ 41.808”	20° 22’ 22.386”	214	415	8	3.1
	LS5	Režiser	44° 47’ 4.031”	20° 23’ 13.704”	336	440	11	1.8
	LS6	Pedaline	44° 47’ 15.029”	20° 24’ 5.861”	479	355	25	2.3

on average depth (unpublished information from a sign placed at the lake shore); d) Lake Biogradsko – Montenegro; surface area: 0.23 km^2, max depth 12 m, average depth 4.5 m (Stanković 1975); e) Lake Crno – Montenegro; surface area: 0.5 km^2, max depth 49 m, average depth 17 m (Stanković 1975); f) Lake Sava – Serbia; surface area: 0.9 km^2, max depth 12 m, average depth 4.5 m (Jovanović et al. 2017). These lakes were selected because we expected them to represent a gradient from oligotrophic to eutrophic conditions. In accordance with Dudley et al. (2013), water chemistry and sediment samples were taken at six sites around the perimeter of each lake (Table 1) and macrophytes were recorded at the same sites. The six sites per lake were selected to together represent the typical shoreline conditions in each lake (with respect to shoreline vegetation, land use, etc.), while at the same time aiming at an approximately even distribution of sites around the perimeter of the lake.

Water and sediment chemistry

At each site, water and sediment samples were taken twice, in consecutive spring and autumn seasons. In lakes Ohrid and Prespa, the samples were taken in October 2016 and April 2017, in Lake Lura in June and August 2017, in lakes Biogradsko and Crno in May and September 2017, and in Lake Sava in October 2017 and April 2018. However, sites LL4, LL5 and LL6 in Lake Lura were only sampled in August and sediment samples in lakes Biogradsko and Crno were only taken in September. We measured water chemical parameters commonly related to eutrophication (phosphorus, nitrogen, biological oxygen demand). In addition, we measured sediment total phosphorus content because phosphorus often limits primary production in lakes and aquatic plants may take up phosphorus from the sediment (Barko & Smart 1981).

At each site, a water sample was collected at a few meters distance from the shoreline at approximately 0.5 m depth. Chemical parameters were measured at laboratories accredited after ISO 17025 according to intercalibrated standard procedures. Total phosphorus (TP) was measured spectrophotometrically after digestion of the water sample with peroxodisulfate (ISO 6878:2004 and APHA 4500-P,B,E) or using ICP-OES after digestion with nitric acid (ISO 11885:2007). Total nitrogen (TN) was measured chemically after oxidation with peroxodisulfate (ISO 11905:1997) or using a TOC/TN analyser after thermocatalytic oxidation (EN 12260:2008 and ISO 8245:1999). Biochemical oxygen demand after 5 days (BOD) was measured according to EN 1899:2009. In addition, a sediment sample was collected at a few meters from the shoreline from approximately 1 m depth using a grab, and sediment P content (in mg/kg dry weight) was determined after digestion with nitric acid (EN 16173:2012) by measuring TP in the extract using the same methods as described above for TP in water.

Macrophyte sampling

Submerged macrophytes, i.e. monocotyledonous and dicotyledonous plants as well as charophytes, were surveyed in July 2016 (lakes Ohrid and Prespa), July 2017 (lakes Biogradsko, Crno and Sava) and August 2017 (Lake Lura) in belt transects of approximately 10 m width – perpendicularly to the shoreline – from the upper littoral to the lower vegetation limit. Primary floras and identification guides were Casper & Krausch (1980, 1981) and Krause (1997). Each transect was divided into depth zones: 0-1 m, 1-2 m, 2-4 m, and >4 m depth. Species occurrence was registered in each transect and each depth zone, and the abundance of each species was estimated according to a five-degree scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant).

Development of indicator values

The indicator values of the BMI (Balkan Macrophyte Index) were based on the macrophyte index (MI) of Melzer (1999) and Melzer & Schneider (2001). Based on our own data and on literature information from the Balkans, we adjusted the MI indicator values to Balkan conditions when necessary and added new indicator species. Indicator values range from 1 (indicating oligotrophic conditions) to 5 (indicating highly nutrient enriched conditions). Consistency of our data with the MI was checked by visually inspecting species lists and assessing which species commonly occurred together. In cases where a species was consistently associated with other species having a higher (or lower) indicator value than the indicator value it had itself, we increased (or decreased) its indicator value, to match the indicator values of the other species more closely. In order to avoid overestimating the importance of rare occurrences, which may not reflect optimum growth conditions for a species, we focused on abundances ≥ 2. Indicator values were left unchanged in all cases where our data either agreed with Melzer & Schneider (2001) or where we had insufficient additional information. Indicator values were changed, however, when the evidence in our data and from previous knowledge was sufficiently clear. In addition, we added indicator values for those species where we had sufficient information with respect to their co-occurrence with species with known indicator values. In all the cases when indicator values have been changed or when we have assigned new indicator values, our reasons are explained in detail below.

We based our indicator values on an existing index because data on macrophytes and corresponding water chemistry from Balkan lakes are scarce, such that a *de novo* development of new indicator values for Balkan lakes based on a purely statistical analysis of the relationships between the occurrence of macrophyte species and corresponding water chemistry was not possible. At the same time, earlier experiences with the MI (Melzer 1999) were promising (Trajanovska et al. 2014, 2019). In addition to the MI, we also consulted available ecological information from literature covering Central Europe, e.g. Casper & Krausch (1980, 1981) and Krause (1997). We also consulted available information from Mediterranean countries in Europe, namely the newly developed macrophyte index from Greece

(Zervas et al. 2018). Zervas et al. (2018) used species-specific indicator values from the pan-European intercalibration metric described in Kolada et al. (2014), which were developed from a Nordic and Central European dataset (no data existed from Mediterranean lakes), and added indicator values for emergent macrophyte species (helophytes). We have not included emergent macrophytes into our index because there is a fundamental difference with respect to nutrient uptake between submerged and emergent macrophytes: submerged macrophytes take nutrients from both sediment and water (Barko & Smart 1981), while emergent species have access mostly to sediment nutrients. For this reason, submerged macrophytes indicate the combined nutrient status of the water and the sediment, while emergent species can only react to sediment nutrients.

Data analysis and calculation of the BMI

Because visual inspection did not indicate a skewed distribution of the data, we used Pearson correlations to test the strength of linear relationships between the BMI and measured chemical parameters. Results were accepted as significant at $p < 0.05$ but, given that we only have few data points, we also carefully interpreted p-values < 0.1. To explore if polynomial relationships fitted the data better than simple linear relationships, models were computed using the MASS-package (Venables & Ripley 2002) in R version 2.14.2 (R Development Core Team 2012), with explanatory variables selected through forward selection based on AIC (Akaike information criterion).

The BMI (Balkan Macrophyte Index) was calculated from the estimated plant abundances in each depth zone at each site and from the species-specific indicator values. In the field, plant abundances were estimated in categories from 1 (very rare) to 5 (abundant, predominant). For calculating the BMI, the cubed abundance categories (termed "plant quantities") were used because they better reflected true differences between rare and abundant species (Melzer & Schneider 2001). In other words, for calculating the BMI, the plant quantities 1, 8, 27, 64, and 125 were used instead of the estimated abundance categories 1, 2, 3, 4, 5. The BMI was calculated as

$$BMI = \frac{\sum_{i=1}^{n} Qi * IVi}{\sum_{i=1}^{n} Qi}$$

with BMI = Balkan Macrophyte Index, Qi = plant quantity (1, 8, 27, 64, 125) of species i, IVi = indicator value of species i. Note that indicator values and plant quantities for each depth zone were used for the calculation of the BMI, meaning that if an indicator species occurred in several depth zones of the same site, it was entered several times into the equation.

A reliable assessment of shoreline eutrophication based on the BMI requires a certain minimum macrophyte abundance. Melzer & Schneider (2001) have defined that minimum abundance based on the summed plant quantities of the indicator species occurring at a site, which must be ≥ 64. This corresponds to at least one indicator species occurring with an abundance of 4, or three indicator species occurring with an abundance of 3. However, since macrophyte occurrences in all depth zones are summed up, a single species occurring in three depth zones with an abundance of 3 would be sufficient to calculate a reliable macrophyte index. Based on our experiences, we preferred a more cautious approach and therefore additionally required that at least two indicator species had to occur at a site. In summary, the BMI may be termed reliable if (i) at least two indicator species occur at a site and (ii) the sum of the plant quantities of the indicator species occurring in all depth zones at a site was ≥ 64.

Results

BMI indicator values

Species-specific macrophyte occurrences at all sites in all six study lakes are given in the Appendix (Table S1, see http://www.acta-zoologica-bulgarica.eu/002370_Appendix) and full species names including their authors are given in Table 2. We found only one species (*Myriophyllum spicatum*) in Lake Crno. Lake Lura was entirely devoid of submerged macrophytes, except for an about 5 cm long non-rooting fragment of *Myriophyllum spicatum*. The other four study lakes had abundant macrophyte vegetation, at least at some sites (Table S1).

We changed indicator values of three species compared to the MI (Melzer & Schneider 2001) and proposed new indicator values for four species. Table 2 summarizes the indicator values for the calculation of the BMI and lists the authors of species names.

Species for which indicator values were changed:

Chara contraria

We found *C. contraria* in lakes Ohrid and Sava, growing together with species characteristic for rather nutrient poor conditions – *Chara aspera* (IV 1.5) and *Chara tomentosa* (IV 2.0) but also with species which are tolerant to some nutrient enrichment – *Potamogeton pusillus* (IV 3.5) and *Stuckenia pec-*

Table 2. Indicator values for the BMI. Small caps: changed indicator value compared to Melzer & Schneider (2001); small caps and underlined: new indicator species. To improve readability, authors of species are given in the row below the species name.

Group 1.0	Group 1.5	Group 2.0	Group 2.5	Group 3.0
Chara hispida L.	***Chara aspera*** Detharding ex Willdenow	***Chara tomentosa*** L.	***Chara globularis*** Thuillier	***Chara contraria*** A. Braun ex Kützing
Chara polyacantha A. Braun	***Chara intermedia*** A.Braun	***Chara virgata*** Kützing	***Nitella opaca*** (Bruzelius) C. Agardh	***Chara ohridana*** (Lj. Kostić) Krause
Chara strigosa A. Braun	***Utricularia minor*** L.	***Potamogeton alpinus*** Balb.	***Potamogeton gramineus*** L.	***Chara vulgaris*** L.
Potamogeton coloratus Hornem.			***Potamogeton natans*** L.	***Myriophyllum spicatum*** L.
Utricularia stygia G. Thor			***Potamogeton × zizii*** W.D.J.Koch ex Roth	***Stuckenia filiformis*** (Pers.) Börner
				Potamogeton perfoliatus L.
				Utricularia australis R. Br.
Group 3.5	**Group 4.0**	**Group 4.5**	**Group 5.0**	
Najas minor All.	***Elodea canadensis*** Michx.	***Elodea nuttallii*** (Planch.) H. St. John	***Ceratophyllum demersum*** L.	
Nitella syncarpa (Thuill.) Chevallier	***Hippuris vulgaris*** L.	***Potamogeton compressus*** L.	***Lemna minor*** L.	
Myriophyllum verticillatum L.	***Lagarosiphon major*** (Ridl.) Moss	***Potamogeton crispus*** L.	***Potamogeton friesii*** Rupr.	
Nitellopsis obtusa (Desv. in Lois.) J. Grov.	***Stuckenia pectinata*** (L.) Börner	***Potamogeton obtusifolius*** Mert. & W. D. J. Koch	***Potamogeton nodosus*** Poir.	
Potamogeton berchtoldii Fieber		***Ranunculus circinatus*** Sibth.	***Sagittaria sagittifolia*** L.	
Potamogeton lucens L.		***Ranunculus trichophyllus*** Chaix	***Spirodela polyrhiza*** (L.) Schleid.	
Potamogeton praelongus Wulfen			***Zannichellia palustris*** L.	
Potamogeton pusillus L.				
Vallisneria spiralis L.				

tinata (IV 4.0) (Table S1). In Lake Ohrid, the species occurred together with *Cladophora* sp., an algal taxon which seems to be associated with nutrient input in Lake Ohrid (Schneider et al. 2014). From previous experience, *C. contraria* was found in Lake Ohrid near Gorica where the river Račanska flows into the lake (Trajanovska 2009), at a site where cattle enter the lake, together with *Potamogeton pusillus* (IV 3.5) and *Zannichellia palustris* (IV 5.0). Although *C. contraria* in lakes Plitvice (Croatia) and Plavsko (Montenegro) indeed was found together with *Chara hispida* (IV 1.0), it also commonly occurred with *Chara vulgaris* (IV 3.0), *Potamogeton pusillus* (IV 3.5), and *Myriophyllum verticillatum* (IV 3.5) (Blaženčić & Blaženčić 1986, 1992). Krause (1997) mentioned that *C. contraria* benefits from slight eutrophication, and also Kolada et al. (2014) assigned an indicator value to *C. contraria* which reflected slightly enriched nutrient levels. We therefore increased the indicator value of *C. contraria* from 2.5 to 3.0, reflecting slightly enriched nutrient levels.

Nitellopsis obtusa

We found *N. obtusa* in Lake Prespa, together with species characteristic of enriched nutrient conditions (among others, *Myriophyllum spicatum (IV 3.0)*, *Potamogeton lucens (IV 3.5), Elodea canadensis (IV 4.0)* and *Ceratophyllum demersum (IV 5.0)*; Table S1). In previous studies, we found *N. obtusa* in Lake Ohrid together with *Elodea canadensis* (IV 4.0), *Stuckenia pectinata* (IV 4.0) and *Potamogeton*

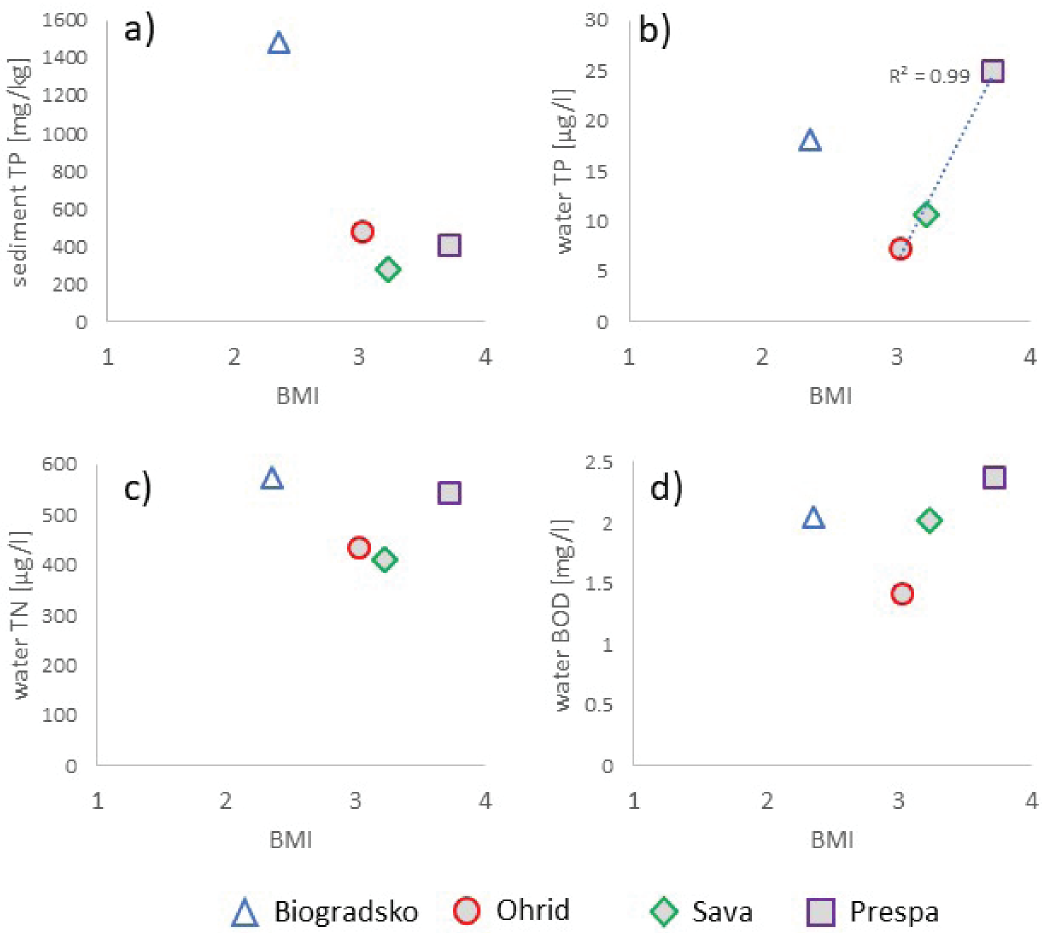

Fig. 2. Average BMI in lakes Ohrid, Prespa, Biogradsko and Sava, plotted against (a) sediment TP, (b) water TP, (c) water TN, and (d) water BOD. Note that the trendline depicted in (b) is based on lakes Ohrid, Prespa and Sava only (excluding Lake Biogradsko).

crispus (IV 4.5) in quite polluted places in shallow water, near the inflow of the river Sateska and in the river Drim close to Struga (Trajanovska 2009, . 2015). Krause (1997) noted that *N. obtusa* tolerates anthropogenic eutrophication "relatively well". Kolada et al. (2014) assigned an indicator value to *N. obtusa* which reflected somewhat nutrient enriched conditions and which was higher (more eutrophic) than the value for *Chara contraria*. We therefore increased the indicator value of *N. obtusa* from 2.5 to 3.5, reflecting more nutrient enriched conditions.

Elodea canadensis

We found *E. canadensis* in lakes Ohrid and Prespa together with species indicating medium to highly nutrient enriched conditions, i.e. *Myriophyllum spicatum* IV 3.0), *Potamogeton perfoliatus* (IV 3.0), *Potamogeton lucens* (IV 3.5), *Potamogeton pusillus* (IV 3.5), *Stuckenia pectinata* (IV 4.0), *Zannichellia palustris* (IV 5.0) and *Ceratophyllum demersum* (IV 5.0). In previous studies in Lake Ohrid (Talevska 2011), we also commonly observed *E. canadensis* together with species indicating only slightly nutrient enriched conditions, such as *Vallisneria spiralis* (new BMI 3.5) and *Chara tomentosa* (IV 2.0). *E. canadensis* in the Balkans has previously been observed in somewhat eutrophic brackish conditions (Bubanja & Stevanović 2013). Casper and Krausch (1980) mentioned that *Elodea canadensis* occurred in eutrophic and – somewhat less abundant – in mesotrophic waters. Kolada et al. (2014) assigned an indicator value to *E. canadensis* reflecting average to high eutrophication. In summary, both our data and published information suggest that *E. canadensis* is common not only in the most eutrophic conditions but also in only slightly nutrient enriched environments. We therefore have reduced the indicator value from 4.5 to 4.0, reflecting that *E. canadensis* occurs with approximately equal abundance at sites with medium to high nutrient enrichment.

Species with assigned new indicator values were:

Chara ohridana

We found *C. ohridana* in Lake Ohrid, where it – in abundances >1 – commonly occurred together

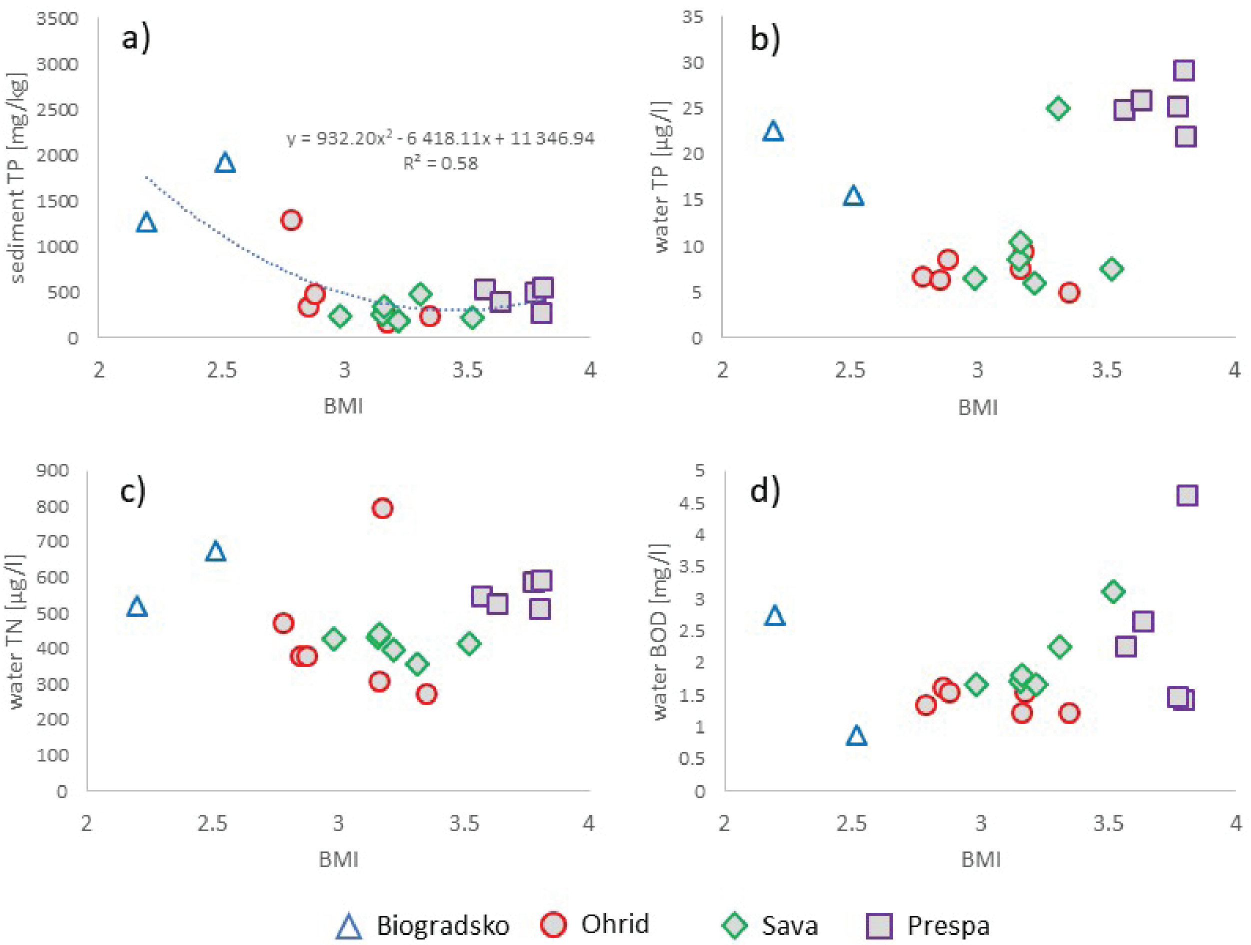

Fig. 3. Site-specific BMI at 6 sites in lakes Ohrid and Sava, 5 sites in Lake Prespa and two sites in Lake Biogradsko, respectively, plotted against (a) sediment TP, (b) water TP, (c) water TN, and (d) water BOD.

with *Chara tomentosa, Nitella opaca, Myriophyllum spicatum, Potamogeton pusillus* and *Elodea canadensis*. Indicator values for these species range from 2.0 (*Chara tomentosa*) to 4.0 (*Elodea canadensis*). Also, the green alga *Cladophora* sp. commonly occurred at places where *C. ohridana* was present (Table S1).*C. ohridana* is regarded as endemic to the Balkans and is registered mainly in lake Ohrid but there are also some records of this species in lake Prespa (Blaženčić & Blaženčić 2002), lake Dojran (Blaženčić & Blaženčić 1999), lake Skadar/Shkodra (Blaženčić & Stevanović 2015, Blaženčić et al. 2018) and one record in Greece (Blaženčić et al. 2006). In previous investigations (Blaženčic & Blaženčić 1999, Trajanovska & Blaženčić 2008, Trajanovska 2009, 2014, Blaženčić & Stevanović 2015, Blaženčić et al. 2018), *C. ohridana* was sometimes registered together with species indicating rather nutrient poor conditions, such as *Chara aspera* (IV 1.5) and *Chara tomentosa* (IV 2.0) but mostly with species indicating slight eutrophication, such as *Nitella opaca (IV 2.5), Chara globularis (IV 2.5), Chara contraria (IV 3.0), Myriophyllum spicatum (IV 3.0), Potamogeton perfoliatus (IV 3.0), Nitellopsis obtusa (IV 3.5), Potamogton lucens (IV 3.5)* and *Potamogeton pusillus (IV 3.5).* There are also some occurrences together with species indicating nutrient enriched conditions, such as *Elodea canadensis (IV 4.0)*, *Stuckenia pectinata (IV 4.0)*, *Ranunculus trichophyllus (IV 4.5)*. However, occurrences with *Zannichellia palustris (IV 5.0)*, a species indicating highly nutrient enriched conditions, were rare. In summary, the available evidence suggested that *C. ohridana* most commonly occurred with species indicating slight eutrophication. Consequently, we assigned an indicator value of 3.0 to *C. ohridana*.

Nitella syncarpa

We found *N. syncarpa* in Lake Prespa occurring together with species having indicator values of 3.0 (*Potamogeton perfoliatus* and *Myriophyllum spicatum*), 3.5 (*Nitellopsis obtusa* and *Potamogeton lucens*), 4.0 (*Stuckenia pectinata* and *Elodea canadensis*) and 5.0 (*Zannichellia palustris* and *Ceratophyllum demersum*). *N. syncarpa* also occurs in lake Skadar/Shkodra, where it has been registered together with other charophytes – *C. virgata* (IV 2.0), *Chara globularis* (IV 2.5), *C. vulgaris* (IV 3.0) and *Nitellopsis obtusa* (IV 3.5) as well as with vascular plants – *Potamogeton perfoliatus* (IV 3.0) and *Vallisneria spiralis* (new BMI IV 3.5) (Blaženčić et al. 2018). Blaženčić & Stevanović (2015) noted that *N. syncarpa* in Montenegro was found in mesotrophic and eutrophic waters. Krause (1997) mentioned that *N. syncarpa* in lake Skadar/Shkodra generally occurred together with *Nitellopsis obtusa* and was often "hiding in muddy sediments". In summary, our data and published information suggested that *N. syncarpa* most commonly occurs together with species indicating somewhat nutrient enriched conditions. Consequently, we assigned an indicator value of 3.5 to *N. syncarpa*.

Vallisneria spiralis

We found *V. spiralis* in lakes Ohrid and Prespa. The species most commonly occurred together with *Myriophyllum spicatum*, a species having an indicator value of 3.0 (Table S1). When presented in abundances of ≥ 3, *V. spiralis* also commonly occurred together with *Potamogeton lucens*, *Potamogeton pusillus*, *Elodea canadensis* and *Stuckenia pectinata*, i.e. species having indicator values of 3.5 and 4.0. More rarely, *V. spiralis* occurred together with species having lower (*Chara tomentosa,* IV 2.0) or higher indicator values (*Zannichellia palustris* and *Ceratophyllum demersum,* IV 5.0 for both species). Casper & Krausch (1980) described that *V. spiralis* occurred together with species indicating medium to high nutrient enrichment (*Elodea*, *Myriophyllum*, *Potamogeton* and *Ceratophyllum* spp.). Altogether, the available evidence suggested that *Vallisneria spiralis* preferentially occurred at somewhat nutrient-enriched locations. We therefore assigned an indicator value of 3.5 to *V. spiralis*.

Najas minor

We found *N. minor* in lakes Ohrid and Sava but the species was most abundant in Lake Sava (Table S1). It occurred mostly together with *Myriophyllum spicatum* (IV 3.0) and *Potamogeton pusillus* (IV 3.5) but also with *Stuckenia pectinata* (IV 4.0) and, rarely, with *Elodea nuttallii* (IV 4.5) and *Zannichellia palustris* (IV 5.0). Lakušić & Pavlović (1976, 1981) and Blaženčić & Blaženčić (1983) found *N. minor* occurring together with *Myriophyllum spicatum, Potamogeton perfoliatus* (IV 3.0) and *Vallisneria spiralis* (new BMI IV 3.5). Casper & Krausch (1980) described that *N. minor* occurred mostly in eutrophic but also in mesotrophic waters. In summary, our data and literature information from the Balkans suggested that *N. minor* commonly occurred together with species characteristic for medium to high nutrient enrichment. We therefore assigned an indicator value of 3.5 to *Najas minor*, indicating somewhat nutrient enriched conditions.

BMI in the study lakes

Macrophytes were sufficiently abundant for calculating a reliable BMI at all six sites in lakes Ohrid and Sava, at five sites in Lake Prespa and at two sites in Lake Biogradsko (Table 3). At the remaining sites, fewer than two indicator species were present and/or the sum of the plant quantities of indicator species was less than 64. From the reliable site-specific BMI values, we calculated an average BMI per lake, which may be used to characterise its trophic status. Lake Biogradsko had the lowest (most oligotrophic) average BMI, followed by – in increasing order – lakes Ohrid, Sava and Prespa (Table 3). Lake Biogradsko had the highest sediment TP content and water TN concentrations among the analysed lakes (Fig. 2). In contrast, Lake Prespa had the highest water TP and BOD concentrations among the study lakes. There were no significant correlations between the average BMI and any of the measured chemical parameters across all four lakes (all $p >> 0.05$). However, when Lake Biogradsko was excluded, the correlation between the BMI and water TP concentrations was marginally supported (Pearson $r = 0.99$, $p = 0.058$; Fig. 2b). Although Fig. 2d suggests that a similar relationship may occur between the BMI and water BOD (if Lake Biogradsko is excluded), it is not statistically supported (Pearson $r = 0.92$, $p = 0.26$).

We also tested for linear relationships between site specific BMI and water and sediment chemistry using the 19 sites from four lakes for which it was possible to calculate a reliable BMI. A significant negative correlation between site-specific BMI and sediment TP content (Pearson $r = -0.60$, $p = 0.006$) was registered but not with any of the measured water chemical parameters (Fig. 3). However, the correlation between site-specific BMI and water TP concentrations was marginally significant (Pearson $r = 0.40$, $p = 0.09$). Visual inspection of Fig. 3a suggested that the relationship between the BMI and sediment TP was more complex. Indeed, model selection based on AIC identified a polynomial model to best describe the relationship between site-specific BMI and sediment TP ($p < 0.001$; see Fig. 3a for model equation and R-squared). The model indicated that up to a BMI of 3.4, an increasing BMI was related to a decreasing sediment TP content. Above a BMI of 3.4, the BMI was related to an increasing sediment TP content (Fig. 3a).

Discussion

BMI in the study lakes

The average BMI (Table 3) ranked Lake Biogradsko as the most oligotrophic among the four studied lakes for which it was possible to calculate reliable BMI values, followed by – in increasing order - lakes Ohrid, Sava and Prespa. Although an average index calculated from only few sites must be interpreted with caution, especially in large lakes such as Ohrid and Prespa, the BMI ranking matches overall expectations. Lake Biogradsko is situated in the Biogradska Gora National Park and is surrounded mainly by forest. Although Filipović & Đurašković (2004) found slightly increased levels of Cadmium in the lake water, Lake Biogradsko generally is subject to little anthropogenic pollution. Lake Ohrid is still mostly considered as oligotrophic, although it clearly is in a process of eutrophication (Matzinger et al. 2007, Schneider et al. 2014) caused mainly by inadequate sewage treatment, tributaries and the subsurface inflow of water from nutrient-rich Lake Prespa (Matzinger et al. 2007). However, the macrophyte vegetation seems to buffer the existing nutrient input into lake Ohrid (Vermaat et al. 2020). Lake Sava has improved in the last decades from a highly eutrophic lake having an average TP concentration of 295 µg/l in the 1980s (Perišić et al. 1987) to average TP concentrations around 40 µg/l in 2014 (Jovanović et al. 2017) and 11 µg/l in the present study. Lake Prespa, on the other hand, is in a process of ongoing eutrophication of anthropogenic origin (Matzinger et al. 2006). Concerns about Lake Prespa were raised publicly since the 1990s (Golterman 2001) and TP concentrations increased from around 20 µg/l in the early 1990s to around 30 µg/l in the early 2000s (Matzinger et al. 2006). The later value is consistent with our measurements (Table 1), indicating that the situation in Lake Prespa did not improve.

Despite the intuitive agreement of the average BMI with the known eutrophication history of the study lakes, water TP, TN and BOD concentrations, parameters which commonly are associated with eutrophication, were not correlated with the BMI. In general, water TP concentrations are often used for establishing stressor-response relationships for macrophyte indices (Kolada et al. 2014, Poikane et al. 2019). Furthermore, the MI, on which the BMI has been based, correlates with lake water TP concentrations (Melzer 1988, Melzer & Schneider 2001). Figure 2 shows that the absence of a correlation between BMI and water chemistry in our dataset is caused by Lake Biogradsko. Despite being situated in a forested catchment with apparently little anthropogenic pollution, Lake Biogradsko had water TP, TN and BOD concentrations similar to – or higher than – Lake Sava, a similarly sized lake with a well-known eutrophication history. When Lake Biogradsko was excluded, the BMI indeed increased with increasing TP concentration, although the correlation, due to few data points, was only marginally significant (Fig. 2b). The reason why Lake Biogradsko is an outlier likely is related to its water level fluctuations. Lake Biogradsko experiences annual water level fluctuations, which may amount up to several meters (Filipović & Đurašković 2004). Lake level alterations may lead to a dramatic decline in the frequency of occurrence of many macrophyte species (Boschilia et al. 2012) and plant regrowth after periods of low water level must occur from seeds (or oospores for charophytes), or from plant fragments. For this reason, the submerged aquatic vegetation of lakes subject to water level fluctuations is likely to be dominated by disturbance-tolerant species and not necessarily by species, which are best adapted to the recent eutrophication history. In addition, nutrient-rich organic matter from the forest surrounding the lake may accumulate in the lake littoral during periods of low water level. This likely explains the relatively high sediment TP content in the littoral of Lake Biogradsko (Fig. 2a). With re-flooding of the sediments, decomposition of parts of the organic material may lead to increasing nutrient and BOD concentrations in the lake water (Kinsman-Costello et al. 2014). Consequently, water nutrient concentrations in Lake Biogradsko were relatively high, while the macrophyte vegetation consisted of disturbance-tolerant species, such as many charophyte species which can re-grow from oospores or thallus fragments (Skurzynski & Bociag 2011). It is important to note that disturbance tolerant species exist among both "oligotrophic" (e.g. many charophytes) as well as "eutrophic" species (e.g. *Elodea canadensis*). Which macrophyte species (if any) occur in a lake subject to disturbance may also be determined by chance. This means that macrophyte indices in lakes subject to water level fluctuations may indicate "too good" as well as "too bad". Also Lake Prespa experiences lake level fluctuations (Matzinger et al. 2006). However, the changes occur over decades instead of annually, as in Lake Biogradsko. Consequently, the macrophyte vegetation in Lake Prespa, in contrast to Lake Biogradsko, apparently had sufficient time to adjust to current nutrient levels.

Macrophytes can take up phosphorus from

both water and sediment (Barko & Smart 1981). It is therefore intuitive to expect that the BMI would be related to sediment and water phosphorus concentrations. The relationship between the BMI and sediment phosphorus was, however, U-shaped rather than simply linear (Fig. 3a). The increasing BMI with increasing sediment TP above a BMI of 3.4 seems intuitive and may be explained with nutrient enrichment leading to both a macrophyte vegetation adapted to higher nutrient concentrations and increased sediment TP (Schippers et al. 2006). However, the relationship between sediment TP and lake eutrophication is complex, and sediments with high TP concentrations may occur in lakes with nutrient-rich and nutrient-poor water (Trolle et al. 2008). Charophytes can act as phosphorus-sinks in lakes (Kufel & Kufel 2002) and sediment TP-content underneath *Chara hispida* beds (IV 1.0) may be the same as underneath *Stuckenia pectinata* beds (IV 4.0; Schneider & Melzer 2004). We have data from only 19 sites in four lakes and more data are required to reliably quantify the relationship between the BMI and sediment TP. However, based on our data in combination with available literature (e.g. Kufel & Kufel 2002, Schneider & Melzer 2004, Trolle et al. 2008), we speculate that the BMI will be unrelated to sediment TP concentrations in oligotrophic environments, whereas it will be positively related to sediment TP in more nutrient-rich conditions.

Two of our study lakes, Lake Lura and Lake Crno, had a depauperate macrophyte vegetation consisting of only one species, *Myriophyllum spicatum*. We suggest that lake level fluctuations may explain the nearly complete absence of macrophytes from Lake Lura. Water from Lake Lura is directed to a small hydropower plant (own observations), and the lack of terrestrial vegetation around the lake in a zone of several meters above the water surface in summer 2017 was an obvious sign that the lake experienced significant water level fluctuations. In contrast, water level fluctuations alone are not sufficient to explain the depauperate macrophyte vegetation in Lake Crno. Lake Crno is located in Durmitor National Park designated a world heritage site in 1980 and protected by legislation of the Republic of Montenegro (Radulović et al. 2010). Lake Crno consists of two basins, which are connected by a narrow strait. This strait dries up during summer, creating two separate bodies of water and leaving the smaller but deeper basin without inflow. This may lead to several meters difference in lake level between the two basins (own observations). While the lake level decrease in summer may explain the absence of macrophytes at sites 5 and 6, which were located in the smaller basin, the larger basin did not show obvious signs of recent major fluctuations in lake level. We observed *Myriophyllum spicatum* at several sites in the large basin, also in shallow water (Table S1). *Myriophyllum spicatum* was, however, the only species of submerged macrophytes found in the entire lake by us. The occurrence of only a single indicator species was the reason why it was not possible to calculate a reliable BMI in Lake Crno. In contrast, earlier studies documented abundant macrophyte vegetation in Lake Crno, also in its small basin, with 18 recorded macrophyte species, six of them being charophytes (Blaženčić & Blaženčić 1994). During our macrophyte survey in 2017, we observed several individuals of crayfish in the lake. Crayfish are known to feed on macrophytes. However, *Myriophyllum spicatum* is known to be "distasteful" to herbivores and crayfish have been shown to consume significantly more *Chara* sp. than *Myriophyllum spicatum* (Chucholl 2013). *Myriophyllum spicatum* therefore may dominate the macrophyte vegetation at sites, which are subject to grazing (Vejrikova et al. 2018). We therefore suggest that the reduction of a formerly abundant macrophyte vegetation to only a single species was due to grazing, possibly mainly by crayfish. Charophytes remove significant amounts of phosphorus from the water by permanently storing it together with $CaCO_3$ in the sediment (Kufel et al. 2016). They thereby actively counteract eutrophication. In contrast, nutrients contained in the biomass of other macrophytes, including *Myriophyllum,* are largely released into the water column during winter decomposition of the dead biomass (Banks & Frost 2017). Water TP concentrations in Lake Crno were around 16 µg/l (Table 1), which is higher than in lakes Ohrid, Lura and Sava. This, together with the disappearance of charophytes, which would have the ability to stabilize an oligotrophic status, is a sign that Lake Crno is at risk of eutrophication. A strong reduction of the crayfish population in Lake Crno may enable the re-establishment of charophytes in the lake and help protect its oligotrophic status.

Future perspectives

Lake level fluctuations are a common phenomenon in Mediterranean climate regions; they are known to affect lake water nutrient concentrations (Ozen et al. 2010) and macrophyte vegetation (Beklioglu et al. 2006). In our study, Lake Biogradsko, which experiences annual water level fluctuations of several meters, was an outlier from the relationship between the BMI and water TP concentrations, having higher water nutrient concentrations than indicated by its – generally not very abundant – macrophyte vegetation. However, stress-tolerant species, which are able to quickly re-grow from seeds, oospores or fragments, exist among "oligotrophic" and "eutrophic species" (e.g. many *Chara* spp., *Myriophyllum spicatum*, *Elodea canadensis*). Consequently, the macrophyte vegetation in lakes subject to water level fluctuations may be absent or dominated by species commonly used to indicate nutrient-poor or, alternatively, nutrient-rich conditions. Any macrophyte index, including the BMI, should therefore be applied with caution in lakes subject to regular water level fluctuations. At the same time, the BMI in Lake Prespa, which experiences comparatively slow changes in lake level at a timescale of decades, apparently gave meaningful results. More data are needed to establish a boundary up to which amplitude in lake level fluctuations macrophyte indices can confidently be applied. Our results indicate that annual drawdowns of several meters may be too much. In such lakes, we recommend using diatoms for indicating the ecological status with respect to eutrophication (Kelly et al. 2014) because they generally react faster than macrophytes (Schneider et al. 2012).

The slope of the relationship between the BMI and water TP (Fig. 2b) was steeper than the slope of the relationship between the MI and water TP in South German lakes, and the intercept of the BMI-TP relationship was at relatively lower (nutrient-poorer) levels than the intercept of the MI-TP relationship (Melzer 1988). E.g., according to Melzer (1988) and Melzer & Schneider (2001), a MI of 3.0 corresponds to approximately 20 µg/l TP. In contrast, a BMI of 3.0 represents around 10 µg/l TP (Fig. 2b). This difference may occur because we measured water chemistry in the littoral directly at the sampling sites, while Melzer (1988) used pelagic TP concentrations during autumn circulation. Nutrients accumulated in the hypolimnion during stratification are re-circulated into the water column during lake circulation, and this process may lead to (temporarily) increased nutrient concentrations. This may explain the higher intercept in the TP-MI regression than in the TP-BMI regression. However, we are aware that our dataset is small and that more data from more lakes may lead to changes in the TP-BMI relationship. Because of our small dataset, we were not able to reliably set boundaries for ecological status classes as required by the WFD. However, based on our results, we now can suggest a way forward as to how status class boundaries may be derived; i) water TP concentrations must be measured and the submerged aquatic vegetation must be recorded in more undisturbed lakes, i.e. lakes without major annual lake level fluctuations, using the same methods as described in this manuscript; nutrient-rich and nutrient poor lakes must both be included, to ensure a balanced gradient from oligotrophic to eutrophic lakes; ii) for these lakes, a correlation must be established between the average BMI and water TP concentrations; iii) natural background water TP concentrations must be derived for each lake from existing (old) data, from models such as Vollenweider's equation (see Brett & Benjamin 2008) for an overview over models predicting lake P-concentrations), or from expert knowledge; iv) the BMI which on the TP-BMI regression corresponds to the natural background TP concentration may be used as reference value for a given lake, or for a group of lakes having similar natural background TP concentrations; v) the variability around the TP-BMI regression may be used for setting class boundaries (see, e.g., Schneider 2011). These boundaries must later be intercalibrated between the countries in the Mediterranean geographical intercalibration group and adjusted, if necessary (Poikane et al. 2015). Although this must be verified later using data, there is no *a priori* reason why large lakes with many endemic species, such as Lake Ohrid, should principally deviate from a TP-BMI regression established based on many lakes from the same ecoregion. This means, if a TP-BMI regression is established and natural background TP concentrations are estimated, it will likely also be possible to establish reference values for large lakes like Ohrid, Prespa and Skadar/Shkodra. This had been considered difficult, because these lakes are "unique" and there is a lack of comparable lakes, from which reference conditions could be derived (Noges et al. 2008).

Since "macrophytes and phytobenthos" are treated as one quality element in the Water Framework Directive, there is also a need to develop indices for phytobenthos in Balkan lakes, which later can be combined with macrophytes into an assessment of ecological status for the entire quality element. In our study lakes, we have tested the most commonly applied diatom indices for ecological status assessment in Europe, i.e. the IPS (Coste in CEMAGREF 1982), TDI (Kelly & Whitton 1995, Kelly et al. 2001), SI (Rott et al. 1997), TI (Rott et al. 1999) as well as the TDIL (Stenger-Kovács et al. 2007). The results are yet unpublished but show that all these indices react to littoral eu-

Table 3. Site specific and average BMI in the study lakes. No reliable values for lakes Lura and Crno were obtained.

Lake	Site	BMI (reliable values)	Average BMI
Prespa	LP1	3.80	3.72
	LP2	3.57	
	LP3	3.78	
	LP4	3.81	
	LP5		
	LP6	3.64	
Ohrid	LO1	3.16	3.03
	LO2	2.78	
	LO3	3.35	
	LO4	2.85	
	LO5	3.17	
	LO6	2.88	
Biogradsko	LB1		2.35
	LB2		
	LB3	2.51	
	LB4	2.20	
	LB5		
	LB6		
Sava	LS1	3.16	3.23
	LS2	3.22	
	LS3	2.98	
	LS4	3.52	
	LS5	3.16	
	LS6	3.31	

trophication pressure and may meaningfully be applied in lakes in the Western Balkans.

Annex V of the WFD, which provides the normative definitions of high, good and moderate ecological status, specifies that not only the taxonomic composition but also the abundance of macrophytes are important descriptors of the ecological status of lakes. The BMI reflects the taxonomic composition of macrophytes but does not provide information on their total abundance. Yet unpublished results from our study lakes indicate that macrophyte abundances indeed may affect water nutrient concentrations. Consequently, there is a need to develop a method how to incorporate macrophyte abundance into lake ecological status assessment. Indeed, Hering et al. (2006) have recommended metrics of the following four types for ecological status assessment: composition/abundance, richness/diversity, sensitivity/tolerance and functional. We have here shown that the BMI may be a good sensitivity/tolerance index for Balkan lakes and future analyses will show if a multimetric index, possibly including an element for macrophyte abundance, may provide improved explanatory power. Finally, descriptions of the macrophyte communities that are expected at different ecological status classes may aid the communication with water managers and citizens (Poikane et al. 2018).

In conclusion, we have developed a macrophyte index which is applicable in Western Balkan lakes, and we have shown that the index is related to water phosphorus (rather than nitrogen) concentrations. We have shown that macrophyte indices may not be applicable in lakes experiencing annual water level fluctuations because the macrophyte vegetation in such lakes may be absent or, alternatively, be dominated by "oligotrophic" or "eutrophic" species. We have also suggested a way to develop reference conditions and status class boundaries for the BMI. Until status class boundaries are in place, we suggest using the MI classes given in Melzer & Schneider (2001). Please note that the MI classes must only be used for illustration and do not comply with WFD status class boundaries.

Acknowledgements: We thank several colleagues at the Hydrobiological Institute Ohrid, the Natural History Museum of Montenegro, the University of Belgrade, the Agricultural University of Tirana, the Institute of Hydrometeorology and Seismology of Montenegro, the Norwegian Institute for Water Research and the Norwegian University of Life Sciences for help with field work. Joanna Lynn Kemp is gratefully acknowledged for correcting the English. The project was financially supported by the Norwegian Ministry of Foreign Affairs.

References

Albrecht C. & Wilke T. 2008. Ancient Lake Ohrid: biodiversity and evolution. Hydrobiologia 615: 103-140.

Banks L. K. & Frost P. C. 2017. Biomass loss and nutrient release from decomposing aquatic macrophytes: effects of detrital mixing. Aquatic Sciences 79: 881-890.

Barko J. W. & Smart R. M. 1981. Sediment-based nutrition of submersed macrophytes. Aquatic Botany 10: 339-352.

Beklioglu M., Altinayar G. & Tan C.O. 2006. Water level control over submerged macrophyte development in five shallow lakes of Mediterranean Turkey. Archiv für Hydrobiologie 166: 535-556.

Birk S., Bonne W., Borja A., Brucet S., Courrat A., Poikane S., Solimini A., van de Bund W., Zampoukas N. & Hering D. 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the water framework directive. Ecological Indicators 18: 31–41.

Blaženčić J. & Blaženčić Ž. 1983. Fitocenološka studija zajednica Charetum fragilis Corillion 1957 i Chareto-Nitellopsidetum obtusae J. Blaž. ass. nova kod Plavnice, na Skadarskom jezeru. (Phytocenological study of the community Charetum fragilis Corillion 1957 and Chareto-Nitellopsidetum obtusae J. Blaž. ass. nova at Plavnica, in lake Skadar). Glasnik Republičkog zavoda za zaštitu prirode i Prirodnjačkog muzeja u Titogradu 16: 7-13.

Blaženčić J. & Blaženčić Ž. 1986. Flora i vegetacija algi razdela Charophyta u planinskim jezerima Crne Gore. (Flora and vegetation of algae in the division Charophyta in mountain lakes of Montenegro). Crnogorska Akademija nauka i umjetnosti Glasnik odeljenja prirodnih nauka 5: 187-203.

Blaženčić J. & Blaženčić Ž. 1992. Macrophytes of Prošće and Ciginovac the lakes of Plitvice. Archive of Biological Sciences, Belgrade 44 (3-4): 213-222.

Blaženčić J. & Blaženčić Ž. 1994. Macrophytes of lake Crno jezero on Durmitor mountain (Montenegro). Glasnik instituta za botaniku i botaničke baste Univerziteta u Beogradu 26-27: 77-86.

Blaženčić J. & Blaženčić Ž. 1999. Doiran lake Charophytes. Annual Review. Biology. Faculty of Natural Science and Mathematics. University St. Cyril and Methodius, Skopje. 52: 83-92.

Blaženčić J. & Blaženčić Z. 2002. Rare and threatened species of charophytes (Charophyta) in Southeast Europe. Phytologia Balcanica*,* Sofia 8(3): 315-326.

Blaženčić J. & Stevanović B. 2015. Katalog harofita (Charales) Crne Gore. Crnogorska akademija nauka i umjetnosti, Odjeljenje prirodnih nauka 10: 1-51.

Blaženčić J., Stevanović B., Blaženčić Ž. & Stevanović V. 2006. Red Data List of Charophytes in the Balkans. Biodiversity and Conservation 15: 3445–3457.

Blaženčić J., Kashta L., Vesić A., Biberdžić V. & Stevanović B. 2018. Charophytes (Charales) of Lake Skadar/Shkodra: Ecology and Distribution. In: Pešić V, Karaman G, Kostianoy A. (eds) The Skadar/Shkodra Lake Environment. The Handbook of Environmental Chemistry 80. Springer, Cham, pp. 169-202. https://rd.springer.com/chapter/10.1007/698_2018_265

Boschilia S. M., de Oliveira E. F. & Schwarzbold A. 2012. The immediate and long-term effects of water drawdown on macrophyte assemblages in a large subtropical reservoir. Freshwater Biology 57: 2641-2651.

Brett M. T. & Benjamin M. M. 2008. A review and reassessment of lake phosphorus retention and the nutrient loading concept. Freshwater Biology 53: 194-211.

Bubanja N. & Stevanović V. 2013. *Elodea canadensis* Michx. New species of flora in Montenegro. Natura Montenegrina 12: 7-12.

Casper S. J. & Krausch H. D. 1980. Pteridophyta und Anthophyta: 1. Teil. In: Ettl H., Gartner G. & Heynig H. (Eds.), Süsswasserflora von Mitteleuropa, 23. Gustav Fischer, Stuttgart.

Casper S. J. & Krausch H. D. 1981. Pteridophyta und Anthophyta: 2. Teil. In: Ettl H., Gartner G. & Heynig H (Eds.), Süsswasserflora von Mitteleuropa, 24. Gustav Fischer, Stuttgart.

Chucholl C. 2013. Feeding ecology and ecological impact of an alien 'warm-water' omnivore in cold lakes. Limnologica 43: 219-229.

Coste M., in CEMAGREF 1982. Etude des méthodes biologiques d´appréciation quantitative de la qualité des eaux. Rapport Division Qualité des Eaux Lyon-Agence Financière de Bassin Rhône-Méditerranée-Corse. In French.

Dudley B., Dunbar M., Penning E., Kolada A., Hellsten S., Oggioni A., Bertrin V., Ecke F., Sondergaard M. 2013. Measurements of uncertainty in macrophyte metrics used to assess European lake water quality. Hydrobiologia 704: 179-191.

European Commission (2000). Directive 2000/60/EC Establishing a Framework for Community Action in the Field of Water Policy. European Commission PE-CONS 3639/1/100 Rev 1, Luxembourg.

Filipović S. & Đurašković P. 2004. Chemical characteristics of the Biogradsko lake watershed. Biodiversity of the Biogradska Gora National Park (Monograph 1, Department of Biology of the University of Montenegro), pp. 149-154.

Golterman H. L. 2001. Editorial – a call for help: The Prespa Lakes – a call for help in a distressing situation. Hydrobiologia 450: 3–4.

Hering D., Feld C. K., Moog O. & Ofenbock T. 2006. Cook book for the development of a Multimetric Index for biological condition of aquatic ecosystems: Experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566: 311-324.

Jovanović J., Trbojević I., Simić G. S., Popović S., Predojević D., Blagojević A. & Karadžić V. 2017. The effect of meteorological and chemical parameters on summer phytoplankton assemblages in an urban recreational lake. *Knowledge and Management of Aquatic Ecosystems* 418, Article Number 38.

Karr J. R. 1999. Defining and measuring river health. Freshwater Biology 41: 221-234.

Kelly M.G. & Whitton B.A. 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. Journal of Applied Phycology 7: 433-444.

Kelly M., Urbanic G., Acs E., Bennion H., Bertrin V., Burgess A., Denys L., Gottschalk S., Kahlert M., Karjalainen S. M., Kennedy B., Kosi G., Marchetto A., Morin S., Picinska-Faltynowicz J., Poikane S., Rosebery J., Schoenfelder I., Schoenfelder J. & Varbiro G. 2014. Comparing aspirations: intercalibration of ecological status concepts across European lakes for littoral diatoms. Hydrobiologia 734: 125-141.

Kelly M. G., Adams C., Graves A.C., Jamieson J., Krokowski J., Lycett E.B. & Murraybligh J. 2001: The Trophic Diatom Index: A User's Manual. Revised Edition. Environment Agency, Bristol, 135 pp.

Kinsman-Costello L. E., O'Brien J. & Hamilton S. K. 2014. Re-flooding a historically drained wetland leads to rapid sediment phosphorus release. Ecosystems 17: 641-656.

Kolada A., Willby N., Dudley B., Noges P., Sondergaard M., Hellsten S., Mjelde M., Penning E., van Geest G., Bertrin V., Ecke F., Maeemets H. & Karus K. 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecological Indicators 45: 407-415.

Krause W. 1997. Charales (Charophyceae). In: Ettl H., Gartner G., Heynig H. & Mollenhauer D. (Eds.), Süsswasserflora von Mitteleuropa, Band 18. Gustav Fischer, Jena.

Kufel L. & Kufel I. 2002. *Chara* beds acting as nutrient sinks in shallow lakes - a review. Aquatic Botany 72: 249-260

Kufel L., Strzalek M. & Biardzka E. 2016. Site- and species-specific contribution of charophytes to calcium and phosphorus cycling in lakes. Hydrobiologia 767: 185-195.

Lakušić R. & Pavlović D. 1976. Vegetacija Skadarskog jezera. Glasnik Republičkog zavoda za zaštitu prirode i Prirodnjačkog muzeja u Titogradu 9: 45-50.

Lakušić R. & Pavlović D. 1981. Associations of Lake Skadar Aquatic Vegetation. In: Karaman G. & Beeton A.M. (eds.): The Biota and Limnology of Lake Skadar. University of Veljko Vlahović, Institute of Biological and Medicine Research Titograd, pp. 125-133.

Matzinger A., Schmid M., Veljanoska-Sarafiloska E., Patceva S., Guseska D., Wagner B., Muller B., Sturm M. & Wuest A. 2007. Eutrophication of ancient Lake Ohrid: global warming amplifies detrimental effects of increased nutrient inputs. Limnology and Oceanography 52: 338–353.

Matzinger A., Jordanoski M., Veljanoska-Sarafiloska E., Sturm M., Muller B. & Wuest A. 2006. Is Lake Prespa jeopardizing the ecosystem of ancient Lake Ohrid? Hydrobiologia 553: 89-109.

Melzer A. 1988. Der Makrophytenindex – Eine biologische Methode zur Ermittlung der Nährstoffbelastung von Seen. Habilitationsschrift der TU München, 249 p.

Melzer A. 1999. Aquatic macrophytes as tools for lake management. Hydrobiologia 395/396: 181–190.

Melzer A. & Schneider S. 2001. Submerse Makrophyten als Indikatoren der Nährstoffbelastung von Seen. In: Steinberg C, Bernhardt H, Klapper H (Eds.), Handbuch Angewandte Limnologie, VIII-1.2.1, pp. 1–14. https://doi.org/10.1002/9783527678488.hbal2001002.

Noges P., Kangur K., Noges T., Reinart A., Simola H. & Viljanen M. 2008. Highlights of large lake research and management in Europe. Hydrobiologia 599: 259-276.

Noges P., Argillier C., Borja A., Garmendia J. M., Hanganu J., Kodes V., Pletterbauer F., Sagouis A. & Birk S. 2016. Quantified biotic and abiotic responses to multiple stress in freshwater, marine and ground waters. Science of the Total Environment 540: 43-52.

Ozen A., Karapinar B., Kucuk I., Jeppesen E. & Beklioglu M. 2010. Drought-induced changes in nutrient concentrations and retention in two shallow Mediterranean lakes subjected to different degrees of management. Hydrobiologia 646: 61-72.

Perišić M., Marjanović P. & Mitrović V. 1987. The eutrophication of lake Sava. Water Science and Technology 19: 1269-1273.

Poikane S., Birk S., Bohmer J., Carvalho L., de Hoyos C., Gassner H., Hellsten S., Kelly M., Solheim A. L., Olin M., Pall K., Phillips G., Portielje R., Ritterbusch D., Sandin L., Schartau A. K., Solimini A., van den Berg M., Wolfram G. & van de Bund W. 2015. A hitchhiker's guide to European lake ecological assessment and intercalibration. Ecological Indicators 52: 533–544.

Poikane S., Portielje R., Denys L., Elferts D., Kelly M., Kolada A., Maernetsg H., Phillips G., Sondergaard M., Willby N. & van den Berg M. S. 2018. Macrophyte assessment in European lakes: Diverse approaches but convergent views of 'good' ecological status. Ecological Indicators 94: 185-197.

Poikane S., Phillips G., Birk S., Free G., Kelly M. & Willby N. 2019. Deriving nutrient criteria to support 'good' ecological status in European lakes: An empirically based approach to linking ecology and management. Science of the Total Environment 650: 2074-2084.

R Development Core Team 2012. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radulović S., Laketić D., Popović Z. & Teodorović I. 2010. Towards candidature of the Crno jezero (black lake) (Durmitor, Montenegro) as a high ecological status (Hes) site of the Dinaric Western Balkan ecoregion. Archives of Biological Sciences 62: 1101-1117.

Rott E., Hofmann G., Pall K., Pfister P. & Pipp E. 1997: Indikationslisten für Aufwuchsalgen Teil 1: Saprobielle indikation., Bundesministerium für Land- und Forstwirtschaft, Wien, 73 pp.

Rott E., Pfister P., van Dam H., Pipp E., Pall K., Binder N. & Ortler K. 1999. Indikationslisten für Aufwuchsalgen in österreichischen Fließgewässern. Teil 2 Trophieindikation. Bundesministerium für Land- und Forstwirtschaft, Wien, 248 p.

Schippers P., van de Weerd H., de Klein J., de Jong B. & Scheffer M. 2006. Impacts of agricultural phosphorus use in catchments on shallow lake water quality: about buffers, time delays and equilibria. Science of the Total Environment 369: 280-294.

Schneider S. 2007. Macrophyte trophic indicator values from a European perspective. Limnologica 37: 281-289.

Schneider S. 2011. Impact of Calcium and TOC on Biological Acidification Assessment in Norwegian rivers. Science of the Total Environment 409: 1164–1171.

Schneider S. & Melzer A. 2004. Sediment and water nutrient characteristics in patches of submerged macrophytes in running waters. Hydrobiologia 527: 195-207.

Schneider S. C., Lawniczak A. E., Picinska-Faltynowicz J. & Szoszkiewicz K. 2012. Do macrophytes, diatoms and non-diatom benthic algae give redundant information? Results from a case study in Poland. Limnologica 42: 204-211.

Schneider S. C., Cara M., Eriksen T. E., Budzakoska Goreska B., Imeri A., Kupe L., Lokoska T., Patceva S., Trajanovska S., Trajanovski S., Talevska M. & Veljanoska-Sarafilovska E. 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica 44: 90-97.

Skurzynski P. & Bociag K. 2011. Vegetative propagation of *Chara rudis* (Characeae, Chlorophyta). Phycologia 50: 194-201.

Stanković S. 1975. Planinska jezera Crne Gore. Društvo za nauku i umjetnost Crne Gore, Posebna izdanja, odjeljenje prirodnih nauka, knjiga 5, 228 p.

Stenger-Kovács C.S., Buczkó K., Hajnal É. & Padisák J. 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. Hydrobiologia 589: 141-154.

Talevska M. 2011. Distribution of *Elodea canadensis* in Lake Ohrid. Proceedings of the International Conference on Carstic Water Bodies in Albania, Alblakes 2: 49-53.

Trajanovska S. 2009. Taxonomy, ecology and status of Charophyta flora in the Lake Ohrid. PhD thesis. University St. Kiril and Methody. Faculty of mathematics and science. Institute of Biology. Skopje.

Trajanovska S., Talevska M., Imeri A. & Schneider S. C. 2014. Assessment of littoral eutrophication in Lake Ohrid by submerged macrophytes. Biologia 69: 756-764.

Trajanovska S. & Blazencic J. 2008. Morphological variability, fructification, distribution and ecology of endemic *Chara ohridana* (Kostic) Krause 1997 from Lake Ohrid. JNU Hydrobiological Institute Ohrid 41(1): 15-22.

Trajanovska S., Talevska M., Budzakoska-Gjoreska B. & Trajanovski S. 2019. Macrophyte-based assessment of nutrient pollution of Lake Prespa, Republic of Macedonia. Acta Zoologica Bulgarica, Suppl. 13: 45-49.

Trajanovska S., Talevska M., Budzakoska-Gjoreska B. & Trajanovski S. 2015. Review of the extant researches of the stoneworts (Charophyta) from Lake Ohrid. University 'St. Kliment Ohridski' Bitola. Hydrobiological Institute. Review 43(1): 96-103.

Trolle D., Hamilton D. P., Hendy C. & Pilditch C. 2008. Sediment and nutrient accumulation rates in sediments of twelve New Zealand lakes: influence of lake morphology, catchment characteristics and trophic state. Marine and Freshwater Research 59: 1067-1078.

Vejrikova I., Vejrik L., Leps J., Kocvara L., Sajdlova Z., Ctvrtlikova M. & Peterka J. 2018. Impact of herbivory and competition on lake ecosystem structure: underwater experimental manipulation. Scientific Reports-*UK* 8, Article Number 12130.

Venables W. N. & Ripley B. D. 2002. Modern Applied Statistics with S, Fourth edition. Springer, New York, NY.

Vermaat J. E., Matzinger A., Trajanovska S., Talevska M. & Schneider S. 2020. Nutrient retention by the littoral vegetation of a large lake: can Lake Ohrid cope with current and future loading? Limnology and Oceanography. DOI: 10.1002/LNO.11460.

Zervas D., Tsiaoussi V. & Tsiripidis I. 2018. HeLM: a macrophyte-based method for monitoring and assessment of Greek lakes. Environmental Monitoring and Assessment 190: 326.

Received: 20.08.2019
Accepted: 22.04.2019